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ESTIMATING THE HAZARD OF CHEMICAL SUBSTANCES TO AQUATIC LIFE

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John Cairns, Jr., Virginia Polytechnic Institute
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16. ABSTRACT

A variety of classes of aquatic toxicity tests are presented and discussed in relation to their ability to provide useful estimates of the environmental effects of chemicals or discharges. These classes of tests can be judged and compared numerically by several standards: ability to permit extrapolation to meaningful effect; ease and cost of conducting tests; applicability to response to a wide variety of chemicals; availability of documented detailed procedures; ecological importance and persistence of effect; and scientific and legal defensibility of the procedure. No single class of tests can be expected to be clearly superior to all others, indicating the need for a variety of testing at successive tiers of intensity of study.

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CHAPTER 2
TOXICOLOGICAL EFFECTS

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Introduction to a Discussion of the Use of Aquatic Toxicity Tests for Evaluation of the Effects of Toxic Substances

REFERENCE: Brungs, W. A. and Mount, D. I., **"Introduction to a Discussion of the Use of Aquatic Toxicity Tests for Evaluation of the Effects of Toxic Substances,"** *Estimating the Hazard of Chemical Substances to Aquatic Life, ASTM STP 657,* John Cairns, Jr., K. L. Dickson, and A. W. Maki, Eds., American Society for Testing and Materials, 1978, pp. 15–26.

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KEY WORDS: water pollution, tests, fishes, bioassay, toxicity, macroinvertebrates, water quality, toxicology

The Toxic Substances Control Act (TSCA, Public Law 94-469) states that human beings and the environment are being exposed each year to a large number of chemical substances and mixtures, and some of these may present an unreasonable risk of injury to health or the enviroment. The law further states that adequate data should be developed with respect to the effect of chemical substances and mixtures on health and the environment and that the development of such data should be the responsibility of those who manufacture or process such chemical substances and mixtures.

This law is rather specific in its general recommendations as to testing (Section 4(b)). It states that the Administrator of the U.S. Environmental

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Protection Agency (EPA) should consider the relative costs of the various test methods which may be required and the foreseeable availability of the facilities and personnel to perform the required testing. The health and environmental effects to be studied include carcinogenesis, mutagenesis, teratogenesis, behavioral disorders, cumulative or synergistic effects, and any other effect which may present an unreasonable risk of injury to health or the environment. The characteristics of chemical substances and mixtures for which testing may be required include persistence, acute toxicity, subacute toxicity, chronic toxicity, and any other characteristic which may indicate such a risk. The methodologies to be used include epidemiologic studies, serial or hierarchical tests, *in vitro* tests, and whole animal tests.

Testing protocols have been developed and used by a variety of federal agencies, universities, and chemical manufacturers. Most of these protocols involved mammalian systems for protecting human health. TSCA equally emphasizes the environment and little effort has been expended in this area. However, two examples of testing protocols for effects evaluation do exist for the aquatic environment.

The National Academy of Sciences [*1*][2] presented a report which was directed to all branches of relevant governmental agencies involved in safety assessments and also to those industries concerned with the manufacture, distribution, and use of chemical products. This report on the principles for evaluating chemicals in the environment was the result of contracts with the EPA in anticipation of pending legislation that resulted in the TSCA. The major considerations of the report were to obtain knowledge of and develop answers to the following questions:

1. How much of a potential pollutant will reach the environment and under what conditions?
2. What will happen to it in the environment?
3. What effects will the chemical cause to man and his environment?
4. How will chemicals interact with other substances in the environment?
5. What will be the importance if a chemical causes various effects?

Several areas for investigation and protocols were discussed for environmental toxicology, simulated ecosystems, and field studies of populations and ecosystems, as well as studies of episodic exposures.

The American Society for Testing and Materials (ASTM) Committee E-35 on Pesticides has prepared a draft laboratory testing scheme (Proposed Standard Practice for a Laboratory Testing Scheme to Evaluate Hazard To Non-Target Aquatic Organisms, 1977, see Related Documents section) for aquatic effects evaluation. It outlines in a stepwise scheme the development and utilization of the chemical and physical properties of the pesticide or other substance, its usage and disposal patterns, its persistence and

[2]The italic numbers in brackets refer to the list of references appended to this paper.

potential for bioaccumulation, its toxicity to representative aquatic organisms, and other pertinent information. The testing protocol emphasizes the importance of information feedback and sequential evaluation to eliminate unnecessary testing and to concentrate major test effort in the areas most pertinent to each individual substance.

Within the EPA, the Office of Toxic Substances and the Office of Pesticide Programs are developing testing protocols with the help of in-house and extramural consultants. Such protocols will provide the basic procedures for the data generation required of industry.

This paper will briefly describe a variety of classes of toxicity tests for aquatic organisms and will propose and explain a set of evaluation criteria for determining the usefulness of these tests. Criteria such as availability of methodology, relative cost, meaningfulness, defensibility, etc. are necessary to select the toxicity tests that have a high present potential for determining the effects of toxic substances. These test and evaluation criteria are presented to provide one of the bases for a workshop discussion on the application of aquatic toxicity tests as predictive tools for aquatic safety evaluation.

Discussion

An evaluation of the potential environmental hazards of chemicals demands much more than the results of appropriate testing protocols. The real and potential exposure characteristics in relation to length and degree of exposure, frequency, locations, persistence, and comparable information are needed together with effects data. Some of this information is necessary for selecting specific testing methods. Otherwise, inappropriate or useless data will be generated at the expense of the most meaningful data. A simple example will exemplify our concern for this fact. Typically, experimental exposures are conducted under constant conditions of water quality and toxicant concentration. If the actual environmental exposures are likely to be intermittent or gradations resulting from single dose (spills), then classical testing procedures might not provide meaningful hazard evaluation. Fluctuating or periodic dose exposures are necessary to simulate many real and potential hazards to aquatic ecosystems.

Another consideration of hazard evaluation is the concept of change versus effect. Unless detectable changes in organisms can be correlated with adverse effect, the test can only be useful for screening and leading to other more direct testing. The National Academy of Sciences [*1*] concluded that with animals, changes in the rate of ventilation, heartbeat, or oxygen consumption might be used as indicators of the effects of chemicals. However, considerable caution should be exercised in interpreting the results of tests for sublethal effects because not all change is necessarily adverse or beyond the limits of compensation. Many of these sublethal effects

or changes do not persist as the exposure continues but commonly there is a gradual shift back to control conditions.

Once some extensive thought has been expended, the necessity of a multiple-tier or heirachical protocol, in contrast to comparable testing regardless of potential hazard, becomes apparent. There is a definite need to progress, if early results so indicate, from one level of testing to another with decisions as to direction and complexity of testing based on the results of basic testing and estimated exposure. This should provide the most efficient and economic use of available "facilities and personnel" (Section 4 (b) of TSCA).

The TSCA consistently refers to an unreasonable risk of injury to health or the environment caused by the manufacture, processing, distribution in commerce, use, disposal, or any combination of such activities. There is no mention of injury caused by accidents associated with the manufacture, processing, distribution in commerce, use, disposal, or any combination of such activities. Many of our more obvious environmental problems, such as polychlorinated biphenyl (PCB), carbon tetrachloride, kepone, polybrominated biphenyl (PPB), and dioxins, were not the result of well designed operations. An expectation of carelessness and accidents is necessary to estimate realistic environmental exposure. A hypothetical example would be a liquid chemical intended for a nonaquatic use with no planned discharge to an aquatic ecosystem. If it is to be distributed in commerce in barges, we believe that any hazard evaluation must assume an accident in transport by this means and require appropriate data generation simulating this type of exposure. Such a hazard evaluation might justify and require alternate transport procedures than intended. The only difficulty in considering accidents in developing protocols lies in the possibility of going too far in the direction of unnecessary and uneconomical testing.

One final consideration before discussing classes of testing and their relative usefulness is that no single class will usually provide sufficient knowledge about a potential hazard to make any final decision even within a multiple-tier protocol system. Inevitably, a group of tests from different classes will be necessary at most testing levels from the simplest to the most demanding.

The following classes of aquatic toxicity tests were not limited to freshwater systems but there may be a need for some alterations or additions if unique marine testing procedures are to be incorporated. Also, some specific testing procedures might fit into more than one class and their utility might be greater or less than that of the class itself, but there is always a danger of simplification or generalization.

Acute Lethality

These are the classical tests of fish and invertebrates that generally estimate exposure concentrations that would result in a 50 percent mortality

(LC_{50}). No distinction is being made here as to time limits, such as 24 or 96 h, or some function of minimum mortality rate [*2*].

Cumulative Mortality and Growth

Embryo/larval testing with fish is the most common example with mortality and growth as the usual endpoints. Embryo/larval tests for the teratogenesis are also considered in this class. Growth or bioenergetic tests with fish and invertebrates cover a wide variety of interests. Effects of stress on food conversion efficiency and food selection (predator/prey) are both potentially significant studies although some of the predator/prey techniques may be more appropriate for consideration in the behavior class [*3*].

Chronic

Life cycle or partial life cycle tests with fish and invertebrates have become quite common and typically provide lethality and growth results as well as effects on reproduction or other sublethal responses during the course of testing. For zooplankton species, these tests are frequently conducted for more than one complete life cycle. For some fish species with relatively short life cycles, the tests will cover one complete generation plus a subchronic (embryo/larval) test with the second generation. For other fish species, the chronic test may begin with juvenile individuals prior to gametogenesis in order to reduce testing time. With these species, an embryo/larval test on the progeny is extremely important because it provides the only opportunity to evaluate that life stage. Reproductive effects include spawning, gametogenesis, and hatching success [*4, 5*].

Physiological and Biochemical

This is an extremely broad class with a great variety of specific procedures but generally has encompassed the area of metabolism of a chemical including absorption, distribution, biotransformation, storage, and excretion. This would include specific enzyme inhibition studies such as acetylcholinesterase and adenosine triphosphatase. Hematologic studies can be useful, together with enzyme studies, to evaluate mode of action and target organs. Physiological and biochemical tests are more useful if the exposures are of sufficient length to determine potential adaptability and persistence of the change or effect for this persistence is necessary to determine the significance of the potential effect.

Behavioral

This class of tests is characterized by procedures with very difficult end-

points and requires numerous subjective decisions. An experienced observer is capable of detecting and grading responses in experimental animals from an objective standpoint, while also obtaining valuable impressions of behavioral effects. Since behavior represents an integrated response of the organism, an impairment in the functioning of nearly any system may be reflected as a behavioral change. As a result, sensitive behavioral measures might serve as indicators that some toxic action has occurred and in this sense may become useful quick-screening procedures for significant environmental effects. Two important considerations in behavioral toxicology involve the selection of the organism test system and the monitoring and evaluation of behavioral effects. Other behavior patterns have been studied in aquatic species such as aggression and territoriality, circadian or other types of rhythms, and predator/prey relationships. Small changes in nest building, courtship, or mating rituals induced by toxic substances may result in reduced reproductive success. Similarly, learning, dominance, maternal or parental behavior, schooling, food selection, and migration can have ecological significance. The same qualifications as to relatability to adverse effects apply here as with the physiological and biochemical tests.

Residue

Before discussion of this class of tests, some clarification of terminology is necessary. Bioconcentration is usually considered to be that process by which toxic substances enter aquatic organisms, by gill or epithelial tissue, from the water. Bioaccumulation is a broader term in the sense that it usually includes not only bioconcentration but also any uptake of toxic substances through consumption of one organism. Biomagnification refers to the resultant total process including bioconcentration and bioaccumulation by which tissue concentrations of bioaccumulated toxic substances increase as this material passes up through two or more trophic levels. There are different test procedures and possibly different test species for each of these. Direct or proven indirect procedures for measuring or estimating maximum residue formation (equilibrium) is necessary as well as elimination rates.

Structure/Activity and Partition Coefficient

These are not biological testing procedures, but characteristics which are or can be estimators of biological processes. The biological activity of structurally related chemicals can be correlated to physical parameters of the chemicals. The principal research efforts on structure-activity correlations have been in mammalian and pest toxicology, particularly in the drug and pesticide industries. One common characteristic is the octanol-water partition coefficient as a measure of the lipophilic properties, and therefore

bioconcentration potential, of chemicals. This class of testing is one of the more promising of the future techniques, especially in the development of new chemicals.

In Vitro

Most cell culture research related to aquatic species has been oriented to bacterial and viral disease problems of fish. The greatest potential use of aquatic *in vitro* testing is probably in the area of metabolism studies. Metabolic characterization of representative cells, metabolism of an environmental chemical by these cells, and the effect of an environmental chemical on cell metabolism could provide useful data to assess exposure as well as environmental fate. Direct transfer of results of *in vitro* tests to hazard evaluation may not be appropriate at this time, but the results may be useful in the same manner as used in mammalian toxicology, that is, as an inexpensive screening test leading to *in vitro* test.

Algal Assays

Primary production has been inadequately studied with regard to the environmental hazard of toxic substances. However, there are relatively simple procedures for algal assay and the laboratory use of cultured periphytic or aufwuchs communities for toxicity testing is increasing. For some classes of chemicals, the most sensitive biological community is that composed of primary producers. One advantage of periphytic or mixed phytoplanktonic cultures is that the relative sensitivity of species can be readily observed when the composition of the species is altered during testing [*6*].

Organoleptic

These seldom used but potentially useful procedures have been associated with effluent testing but have a broader applicability. Since one of the justified uses of aquatic resources is the human consumption of part of their productivity, it is appropriate to protect this use by controlling those toxic substances whose sublethal effects may only result in reduced acceptability of aquatic populations for human consumption. This class of testing has some comparability to the residue class, inasmuch as the concern for each is usually with the uptake or organic chemicals. Also, in most instances the hazard (residue or off-flavor) is a greater problem to the consumer than it is to the populations themselves [*7*].

Histological

The usefulness of studies of modifications of tissues and organs derives from evidence that changes in structure may reflect changes in function.

However, structural change alone does not imply functional change. At best, these relationships (much like physiological, biochemical, and behavioral relationships) are only suggestive, and their meaningfulness must be pursued either by additional studies or by a correlative evaluation of the findings in other components of subchronic or chronic toxicity testing [*8*].

Microcosms

At present, most procedures involving laboratory microcosms and artificial laboratory streams are limited in organism sample size and consequently cannot provide meaningful biological effects data. They can provide useful data on the environmental behavior of a toxic substance including its metabolism, degradation, and subsequent movement through aquatic systems. Some efforts have begun in an attempt to incorporate effects testing into some types of microcosms. One of the principal difficulties in this class of tests is the common inability to replicate the baseline characteristics of these ecosystems [*9*].

Field

The basic difficulty in field evaluations is similar to that of microcosm testing, that is, uncontrollable baseline variability. Most biological populations naturally undergo diurnal, seasonal, or annual fluctuations over one or more orders of magnitude. Superimposed on this is a commonly smaller degree of variability due to man-induced sublethal stress. Few investigators have consistently been able to sort these two sources of variability. The variability in recruitment potential or recovery capability (or environmental adaptability) of an ecosystem subjected to stress also contributes to the difficulty of assessment. Nutrient cycling, productivity, and energy flow may need to be studied at times and are unlikely to be modeled adequately in the laboratory. By their nature, field observations and studies are limited to effects evaluation after the fact and can only provide limited predictability.

Diversity

Laboratory and microcosm testing of aufwuchs, periphyton, or similar communities can provide useful predictive measures of environmental effects, and diversity changes may be one of the endpoints of concern. Field diversity measurements are subject to the same difficulties and qualifications of field studies in general. As with many effects-testing procedures, decisions have to be made as to what level of reduction in diversity is unacceptable.

Benthic

Recent legislation on disposal of dredged materials and ocean dumping

has demonstrated a real need for testing procedures that can provide an analysis of the potential impact of these solids. These regulations or subsequent guidelines will specify benthic toxicity tests to estimate the potential hazard of disposal of these waste materials. As these procedures evolve and are developed, they will probably fit in some of the previously discussed classes of tests.

Evaluation

The various classes of aquatic toxicity tests can be evaluated for their relative ability to provide useful and meaningful estimates of environmental hazard. Table 1 is a form to be used in this evaluation. The evaluation criteria should be ranked from 1 to 6 with a numerical value of 1 to 10 for each class of tests under each criterion. A summary ranking will show the present potential for use. Also, this evaluation is based on present conditions and, hopefully, will be more meaningful as test methods are refined, improved, or developed. A class of tests may receive a low rating overall or in a single category. There may be a test in that class that for some unique reason provides good effects estimates. Consequently, it is necessary to use judgment when developing a testing protocol. TSCA will result in a classification or categorization of chemical substances or mixtures (Section 26(c)). Each class may have its own protocol. This categorization may be based on similarity in molecular structure, in physical, chemical, or biological properties, in use, or in mode of entrance into the human body or into the environment, or some other system of classification.

Simplicity and Cost

Section 4(b)(1) of TSCA requires that, in determining the testing requirements, the Administrator of EPA shall consider the relative costs of various test protocols and methodologies as well as the reasonably foreseeable availability of the facilities and personnel to perform the testing.

Extrapolation

A test for hazard evaluation must either be a direct measure of some ecologically significant effect, such as reproduction, avoidance, or unacceptable residue, or it must permit a valid extrapolation to such an effect. This problem of justifiable extrapolation is one reason why we still have to conduct expensive, high risk, chronic toxicity tests for the development of environmental criteria.

Chemical Class Specificity

Some tests, such as acute lethal, would be applicable to most chemical

TABLE 1—*Evaluation of classes of simple and complex toxicity testing for evaluation of effects.*

Class of Testing	Simplicity and Cost	Extrapolation	Broad Applicability for Chemical Categories	Ecological Importance of Effect	Availability of Routine Methods	Scientific and Legal Defensibility	Present Potential for Use
			Simple Systems				
Lethality							
Embryo/larva							
Reproduction							
Physiological and biochemical							
Behavioral							
Residue							
Structure/activity and partition coefficient							
In vitro							
Algal assay							
Organoleptic							
Histological							
			Complex Systems				
Microcosm							
Field							
Diversity							
Benthic							

substances or mixtures, although water solubility differences for some might preclude meaningful comparisons. Organoleptic tests have a more limited applicability since only a few categories of chemicals would be expected to cause off-flavor in aquatic species. A test of broad applicability would, generally, be a more important or useful test.

Ecological Importance of Effect

Mortality is obviously important, but what of a reduction in growth? Is avoidance more ecologically risky than an increase in tumor formation in fish exposed to industrial pollution? These and similar questions must be considered and answered before establishing the most appropriate test protocol. An additional factor for consideration is whether the effect is on a single species or all populations, on a single ecosystem or all ecosystems.

Availability of Routine Methods

Testing protocols should cite published, detailed test procedures so that these parties responsible for the conduct of the tests will provide consistent, high quality data. Consequently, newer, potentially useful, procedures may not rank as high now as they would in a few years. This is an indication as to immediate needs for methods development.

Scientific and Legal Justification

Most regulatory decisions involving the establishment of standards are eventually subject to scrutiny on their scientific and legal justification. When testing protocols for categories of chemical substances or mixtures are finalized under TSCA, there will be a need for extensive defense of those protocols. If those responsible for the protocol cannot defend its content or implications, another round of protocol development will be necessary. Data requirements must be designed specifically to assist in determining the possibility of "an unreasonable risk of injury to health or the environment" (Section 4(a) of TSCA). It is unlikely that data requirements beyond this will be justifiable.

Summary

The final evaluation criterion (Table 1) for present potential for use is superficially like a priority sequence of testing; it should be more than this. It should indicate weak spots in our research programs as well as guide us in developing the research programs for developing and evaluating screening and monitoring techniques that are "rapid, reliable, and economical" for "carcinogenic, mutagenic, teratogenic, and ecological effects

of chemical substances and mixtures" (Section 10(c) of TSCA). This criterion for present potential must not lock us into a stagnant mode; if it does, this evaluation will have been counterproductive.

We hope the workshop attendees will find this evaluation system as a means to develop the most concise and predictive procedures for determining the potential effects of toxic substances on aquatic ecosystems.

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